# VARIATIONS IN THE OSMOTIC CONCENTRATION OF THE GUARD CELLS DURING THE OPENING AND CLOSING OF STOMATA

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It has been repeatedly shown that the opening and closing of stomata are closely related to the turgor of the guard cells. The first critical study upon this point was made by von Mohl (1856). Following von Mohl, work has been done by Schwendener (1881), Kohl (1886), Francis Darwin (1898), Leitgeb (1886), and Lloyd (1908). These investigators state that the opening and closing of the stomata are due to variations in the turgor of the guard cells, and that the condition of the turgor of these cells depends, first, upon the abundance of water in the plant, and second, upon the amount of effective osmotic substances in the cells. It was supposed until recent researches that whenever a plant was not well supplied with water, or in other words in a slightly wilted condition, the stomata would always be closed. Many of the earlier investigators showed to their own satisfaction that whenever the plant begins to wilt the stomata invariably close. In more recent years, Laidlow and Knight (1916) and Lloyd (1912, 1913) have contradicted this statement after finding stomata still open when plants had become badly wilted.

The second factor upon which the turgor of the guard cells depends, that of the amount of effective osmotic substances, has not been given so much attention. The observations made by Lloyd (1908) and Darwin (1898) on the starch content of the guard cells as compared with the other tissues of the leaves, and those made later by Iljin (1914), are the most important. Lloyd found that the starch content in the guard cells is greatest in the early hours of the morning and gradually disappears until the time when the stomata are at their maximum width. After the stomata begin to close there is a gradual accumulation of starch until the maximum is again reached at some hour during the night. Lloyd also found that the fluctuation of the amount of starch within the plastids of the guard cells is accompanied by a complementary fluctuation of the oil content.

Iljin (1914) made similar observations on the starch content of the guard cells of several plants which he had under study. The important phase of his work, however, was the actual determination of the differences in osmotic concentration between the guard cells of the stomata and the cells of the other tissues of the leaf. For the purpose of determining the threshold concentration, he used concentrations of $KNO_3$ varying in strength from 0.125 to 3.00 normal. His experiments in general showed an extra-

ordinarily high osmotic concentration for the guard cells when the stomata are open as compared with the osmotic concentration in the epidermis and leaf parenchyma. He found little or no difference between the osmotic concentration of the guard cells and that of the epidermis of the leaf parenchyma when the stomata are closed. The latter tissues always remained constant in their osmotic pressures. Table 1 will serve to illustrate the extreme differences in osmotic concentration which Iljin found between the guard cells and the parenchyma of the leaves.

TABLE 1

| Name of Plant | Pressure in Atmospheres: Guard Cells | Parenchyma |
|---|---|---|
| *Senecio Doria* | over 80 | 22.5 |
| *Senecio Doria* | 108 | 22.5 |
| *Centaurea orientalis* | 53.7 | 21.4 |
| *Centaurea orientalis* | 98 | under 24 |
| *Iris pumila* | 90 | " 24 |
| *Iris pumila* | 98 | 13 |
| *Eryngium campestra* | 98 | 19.1 |
| *Verbascum Lynchnitis* | 80.5 | 17.9 |
| *Veronica incana* | 90 | 45(?) |

The concentration in all cases is expressed as pressure in atmospheres. The greatest difference in concentration between the guard cells and the parenchyma always occurred when the stomata were open to the greatest width. No determinations were made before the stomata were open in the morning, but the observations made at 8 a.m., 12 noon, 4 p.m., and 7:30 p.m. showed a marked decrease in the difference in osmotic concentration from 8 a.m. to 4 p.m., at which latter time the concentration was the same in all tissues.

Iljin does not explain his method of calculating osmotic concentration in atmospheres of pressure. However, the osmotic concentrations reported in this paper will be calculated according to the tables and formula given by Jones (1907). All of Jones' calculations of osmotic concentration are based on the depression of the freezing point and the concentration of the solution. He gives table 2 for calcium chloride.

TABLE 2

| Concentration | Lowering of Freezing Point | Atmospheric Pressure |
|---|---|---|
| .102 mol. | .505 | 6.08 |
| .153 | .752 | 9.054 |
| .204 | 1.012 | 12.184 |
| .255 | 1.267 | 15.255 |
| .306 | 1.537 | 18.505 |
| .408 | 2.104 | 25.332 |
| .510 | 2.681 | 32.279 |
| .612 | 3.348 | 40.31 |
| 1.000 | 6.345 | 76.394 |

The third column is calculated from Jones' formula for osmotic concentration of solutions expressed in atmospheres of pressure. The formula is as follows:

$$\text{Osmotic concentration of a solution expressed in atmospheres of pressure} = \text{Freezing point depression of that solution} \times \frac{22.4 \text{ osmotic concentration of a molecular solution expressed in atmospheres of pressure}}{1.86 \text{ freezing point depression of a molecular solution}}$$

In order to check the strength of the solutions used in the following experiments with those of Jones, the freezing-point depressions of three concentrations used were determined with the aid of the Beckman apparatus. These determinations checked very closely with those given by Jones for solutions of the same strength. Thus it was considered safe to use his results for all calculations.

The experiments to be reported in this paper were made with a view to securing more data on the specific problem of the variations in osmotic concentration (1) of the guard cells during the opening and closing of the stomata, and (2) of the guard cells as compared with the other cells of the epidermis.

## Methods

The same general method was used throughout the various tests herein recorded. A series of concentrations of $CaCl_2$[1] was made up varying in strength from 1.00 molecular to 0.06 molecular by dilutions from a stock solution of gram-molecular concentration. The respective concentrations were: 1.0; 0.8; 0.6; 0.5; 0.45; 0.40; 0.35; 0.30; 0.28; 0.26; 0.24; 0.22; 0.20; 0.18; 0.16; 0.14; 0.12; 0.10; 0.08; 0.06 molecular. This series was determined upon after some preliminary trials. In making tests for the threshold concentrations of the guard cells and also of the epidermal cells (both determinations being made at the same time) portions of the epidermis were removed from the lower surface of the leaf and placed on microscopic slides. Two drops of the solutions of varying concentrations were then placed on each section. These prepared slides were then placed under moist chambers for a period of five to ten minutes. At the end of this period the sections of epidermis were covered with cover glasses and examined microscopically for plasmolysis both of the guard cells and of the cells of the epidermis. In this way a complete series could be tested and examined in a comparatively short time. Flat petri dishes moistened with the various solutions were used as moist chambers for the slides to prevent evaporation and a consequent change in the concentration of the solution.

[1] $CaCl_2$ was used in these experiments in preference to $KNO_3$ because of the tendency of the latter to make the protoplasm more permeable.

## Material

By preliminary tests, it was found difficult to determine when epidermal cells were plasmolyzed if they did not contain pigment. This made it necessary to choose plants possessing pigmented cells in the lower epidermis. It was fairly easy to determine when guard cells were plasmolyzed on account of the density of their protoplasm. The plants found best for making quick and accurate determinations were Cyclamen, Iresine, *Zebrina pendula*, and young beet. The leaves of all these plants possess pigmented cells in the lower epidermis. The epidermis may be separated rather freely from the leaf parenchyma. This fact increases the rapidity with which mounts and determinations can be made. The work was begun in the laboratory of the Department of Plant Physiology at Cornell University at the suggestion of Dr. Lewis Knudson, and carried to completion in the laboratories at the University of Missouri. The same methods were followed at both places, and as far as possible the same kinds of plants were used in the tests.

## Results of Tests

### *Zebrina pendula*

The first tests recorded on *Zebrina pendula* were made on November 27. The results are shown graphically in figure 1. The first observation was

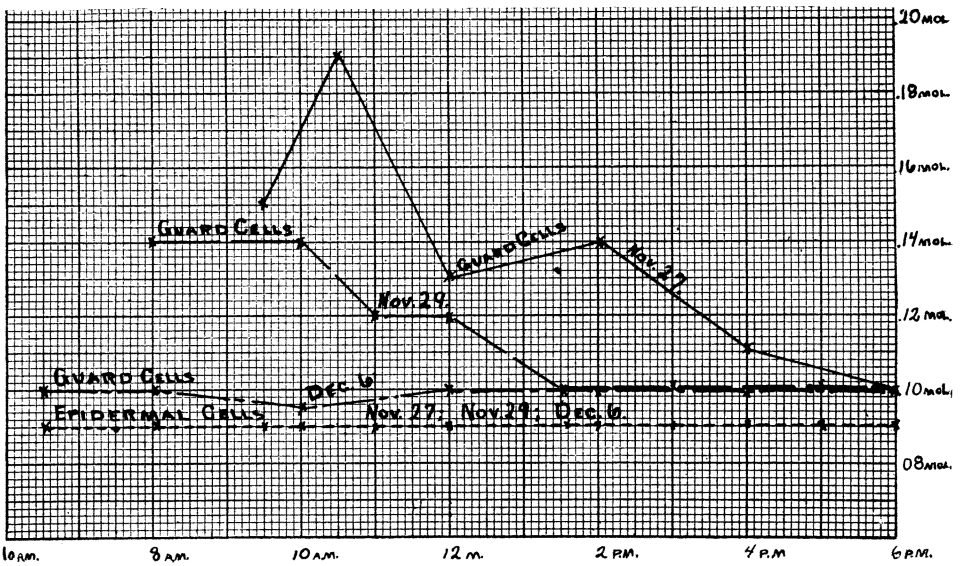


FIG. 1. *Zebrina pendula.* Observations made at Ithaca, N. Y.

made at 9:30 a.m. At 10:30 a.m. the highest osmotic concentration of the guard cells was observed. From this hour to sundown there was a gradual decrease. On the other hand, the threshold concentration of the epidermal

cells remained the same throughout the day.[2] The greatest difference in osmotic concentration expressed in atmospheres was 5.97. It should be said in regard to the threshold concentration that it is difficult to determine exactly what concentration is the threshold, since all cells are not equally affected. The threshold concentrations in these tests were always considered the points at which the slightest plasmolysis could be detected, For these reasons there is bound to be some error in the determinations, but when differences in the concentration are large there is little difficulty in making observations. It is worthy of notice that the difference between the guard cells and the cells of the epidermis is very small as compared with the results secured by Iljin, although there is a rise with the opening of the stomata and a fall with their closing. The sun was visible 0.2 of the time from 8 to 9 a.m., and almost constantly from 9 a.m. to 12:30 p.m., after which there was no sunlight.

Further observations were made on *Zebrina pendula* on November 29, December 5, and December 6. The results of these observations are also shown graphically in figure 1, with the exception of those of December 5, when no differences were found. On November 29 the sun came out at 9:30 a.m. and was fairly bright the rest of the day. On December 5 there was practically no sunshine throughout the day. On December 6 the sun did not shine until 9:30 a.m., after which there was almost constant sunshine. On dark days there was very little if any opening of the stomata, although some stomata were always found open regardless of the time at which observations were made. On bright days there was a rather ready response of the stomata. They were usually open to their maximum by 10–11 a.m.

No observations were made on this plant at Columbia, Missouri, because no vigorous plants were available.

*Cyclamen*

The first recorded tests on cyclamen were made on November 29, when the sun shone most of the time after 9 a.m. The results are given in figure 2. There was no great difference between the osmotic concentration of the guard cells and that of the cells of the epidermis at any time. The greatest difference recorded was only a little over three atmospheres. The curve, however, indicates a rise in the osmotic concentration in the morning and a fall in the afternoon in the case of the guard cells (observations made at Ithaca).

The second series of observations was made on December 6, when the sunshine was rather constant after 9:30 a.m. These results are in almost perfect accord with those given for November 29 (observations made at Ithaca).

[2] The threshold concentration of the epidermal cells was found to be constant in all the experiments. Only slight variations in the readings occurred in every case. For this reason the threshold concentration of the epidermal cells is given as a straight line in the graphs.

Figure 3 shows the results of observations on cyclamen made on January 1 and January 2, 1918, at Columbia, Missouri. Here a new but entirely similar series of concentrations of $CaCl_2$ was used. The results recorded

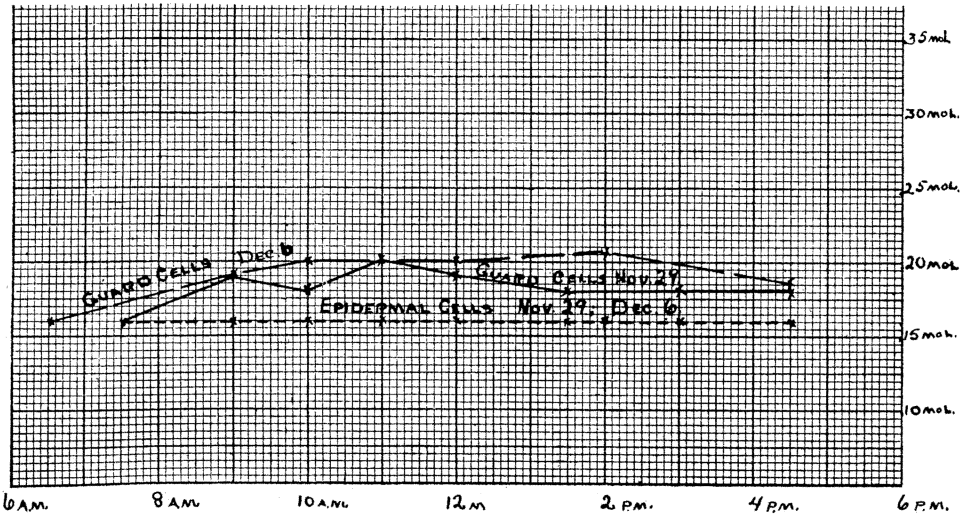


FIG. 2. Cyclamen. Observations made at Ithaca.

here were secured after several days of preliminary work with the new plants, apparatus, and solutions in order to make the error as small as possible.

It is quickly seen that these results are very different from those secured at Ithaca. There is a rise in osmotic pressure in the morning and a fall in

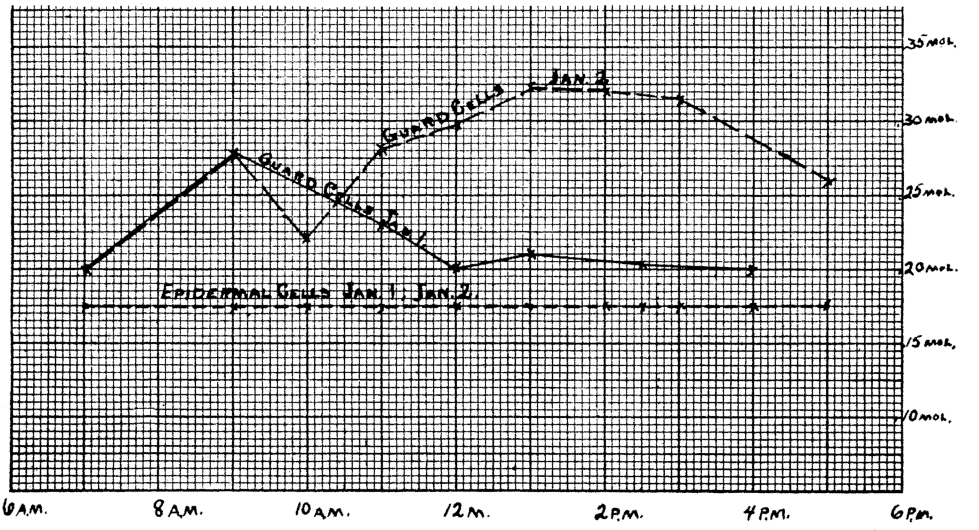


FIG. 3. Cyclamen. Observations made at Columbia, Mo.

the afternoon, as was indicated by the data secured at Ithaca, but the difference between the osmotic concentration of the guard cells and that of the cells of the epidermis is much greater. Expressed in atmospheres, the difference is 8.72 as compared with 3.00 at Ithaca.

The difference between the curves of January 1 and January 2 may be explained by the difference in the amount of sunshine. On January 1 the sun did not shine during the entire day, while on January 2 the sunshine was continuous in the afternoon although very limited in the forenoon.

Figure 4 shows a check series run with $KNO_3$ instead of $CaCl_2$. The

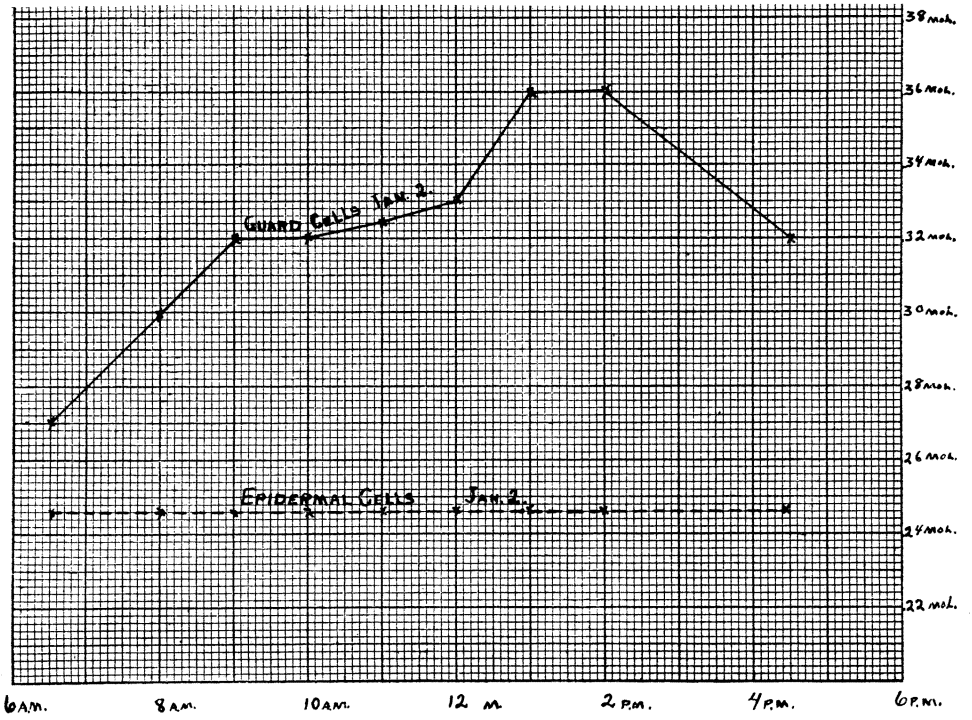


FIG. 4. Cyclamen. Observations made at Columbia.

same general curve is secured with these solutions as with solutions of $CaCl_2$; however, the molecular concentration in all cases was considerably higher. This is probably shown best by a comparison of the threshold concentrations of the epidermal cells in the two series.

Figure 5 shows the results of observations on cyclamen made at Columbia on January 3. The sun shone brightly until 1:45 p.m., after which there was no sunlight. These results accord with the results of January 2, with the exception that there was a greater difference between the osmotic concentrations of the epidermal and of the guard cells. Expressed in atmospheres, the greatest difference was 19.4.

The second curve shows the results of observations made on a check plant. With this exception all other tests at Columbia with cyclamen were made on the same plant. The perfect accord between these two plants, however, seems sufficient proof that the first plant was a normal one.

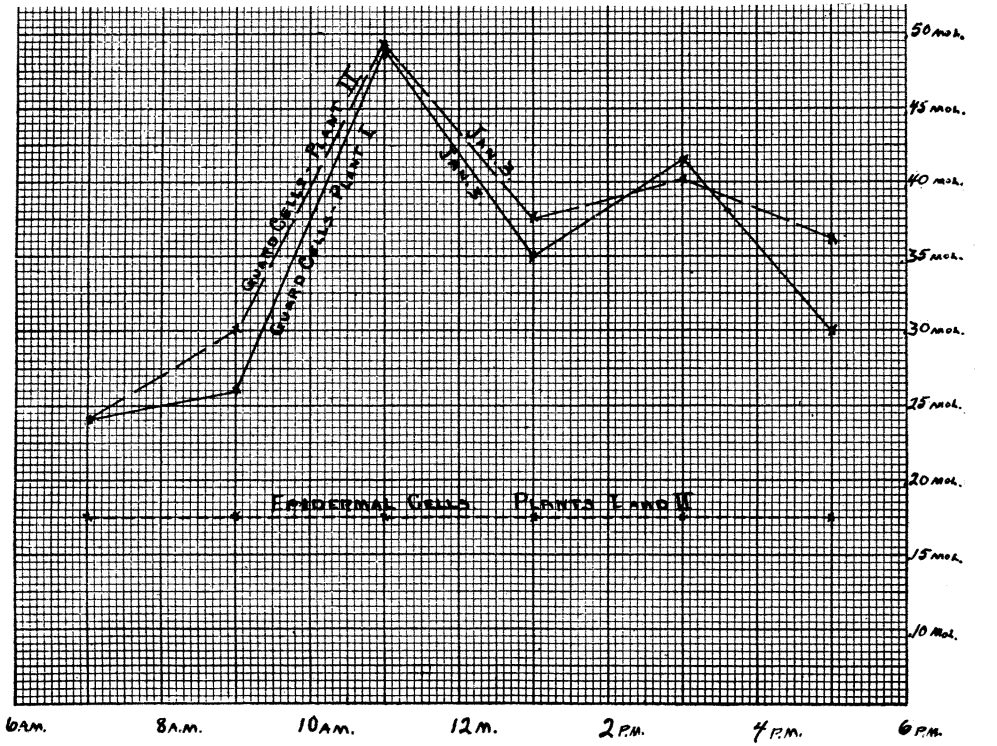


FIG. 5. Cyclamen. Observations made at Columbia.

*Iresine*

Figure 6 gives the results of observations on Iresine in a graphic manner. Curve (*a*) shows a rise in the osmotic concentration in the forenoon and a subsequent fall in the afternoon, with a maximum difference between the osmotic concentrations of the guard cells and the cells of the epidermis of 6.78 atmospheres. Curves (*b*) and (*c*) show a much greater rise in the forenoon and consequently a much greater fall in the afternoon than curve (*a*). Likewise the difference between the osmotic concentrations of guard cells and cells of epidermis is greater. The maximum difference amounted to 28 atmospheres.

*Beet*

Figure 7 shows the results of observations made on young beet plants at Columbia, Missouri, on January 4, when the sunshine was fairly constant

throughout the day. The same general results were secured with this plant as with the other plants studied under similar environment. The greatest difference between the osmotic concentrations of the epidermal and the guard cells is 19 atmospheres' pressure.

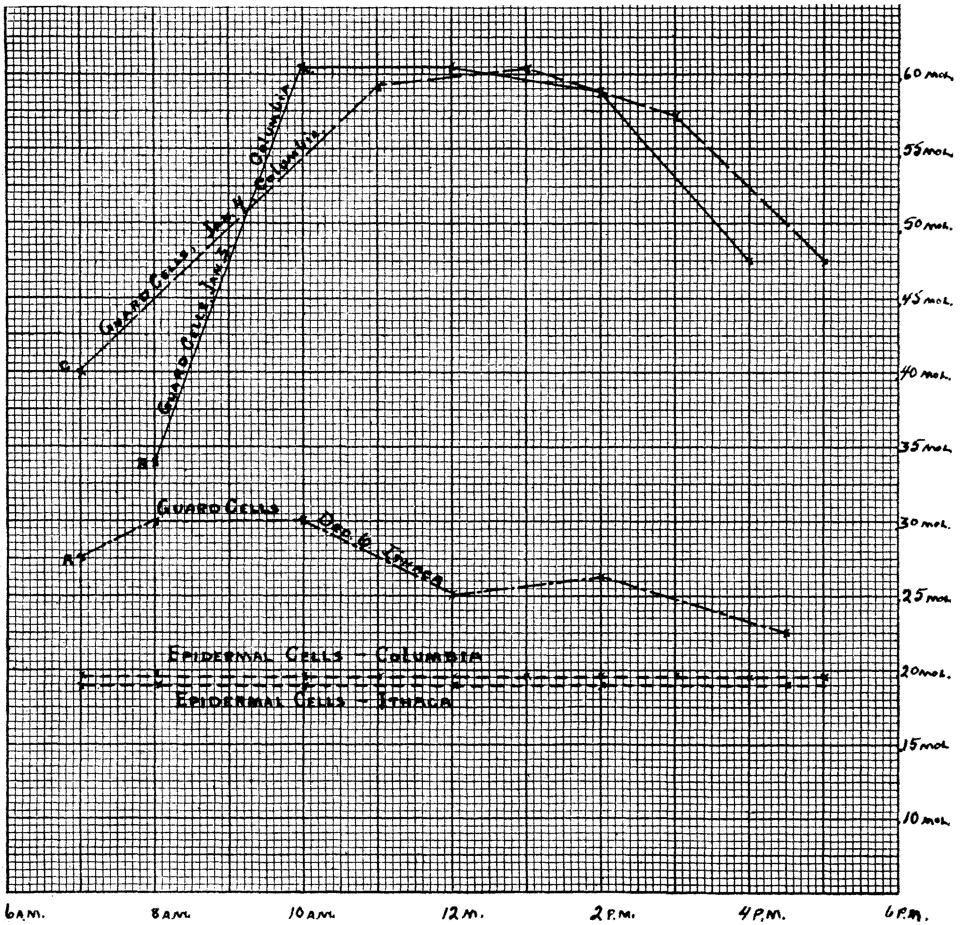


FIG. 6. Iresine.

## SUMMARY

The experimental data given in the foregoing charts on the whole do not show very great differences in osmotic concentrations between epidermal and guard cells. These differences may be summarized as in table 3.

These results are not in entire harmony with the results of Iljin, since the greatest difference here shown in osmotic concentration between guard and epidermal cells only approaches the smallest difference secured by Iljin.

The variations between the results at Ithaca and those at Columbia are difficult to explain, but may be due in part (1) to the individual plants

employed in the experiments, (2) to the greater amount of sunshine at Columbia, and (3) to the greater temperature and humidity of the greenhouse in which the work was carried on at Columbia. There was never a time when the stomata were found completely closed at Columbia; most of the time they were found wide open. (On account of the equipment at Columbia it was impossible to determine the condition of the stomata at night.)

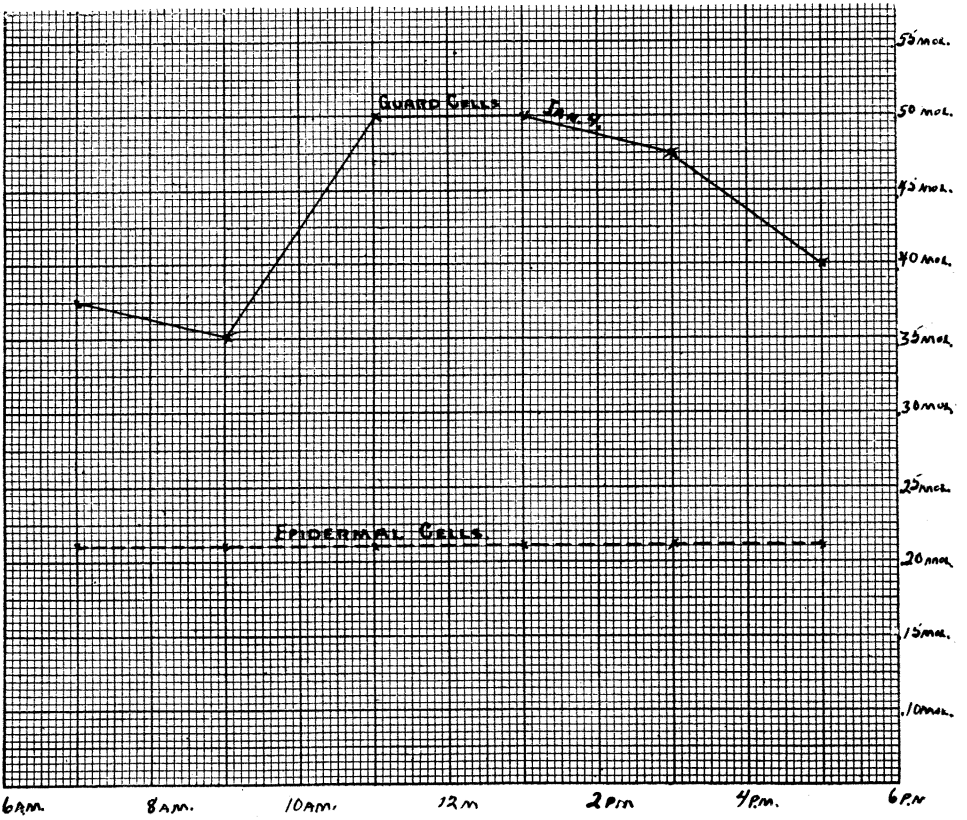


FIG. 7. Young beets. Observations made at Columbia.

TABLE 3

| | Osmotic Concentration Expressed in Atmospheric Pressure | | | |
|---|---|---|---|---|
| | Ithaca, New York | | Columbia, Missouri | |
| | Epidermis | Guard Cells | Epidermis | Guard Cells |
| *Zebrina pendula* | 5.35 | 11.32 | | |
| Cyclamen | 9.48 | 12.55 | 10.09 | 29.49 |
| Iresine | 11.33 | 18.11 | 11.63 | 39.52 |
| Beets | | | 12.55 | 31.50 |

The conclusions to be drawn from these experiments are:

(1) That there is a difference between the osmotic concentration of the guard cells of the stomata and that of the other epidermal cells when the stomata are open.

(2) That the osmotic concentration in the guard cells increases in the early hours of sunshine and decreases in the afternoon, approaching the osmotic concentration of the epidermal cells at nightfall.

(3) That there is very little if any change in the osmotic concentration of the epidermal cells during the day.

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